ACUTE TOXICITY OF WATER EXTRACTS OF BARK OF THE NEEM PLANT, AZADIRACHTA INDICA (LODD) TO THE AFRICAN RIVER PIKE HEPSETUS ODOE (SARCODACES) ODOE (BLOCH)

BY Oti Emmanuel Egwu
College of Natural Resources and Environmental Management
Michael Okpara University of Agriculture
Umudike, P.M.B. 7267, UMUAHIA, ABIA STATE, NIGERIA

ABSTRACT

The effects of the water extract of bark of the Neem plant (Azadirachta indica) on the mortality rate, opercular ventilation rate, and some behavioural respionses of H. odoe were investigated under laboratory conditions over a 96 h exposure period. The 96 h LC50 waqs determined as 8.00mg L-1. The extract led to an initial increase in the opercular ventilation rates which then decreased significantly ($P<0.05$). Prior to death, darkening of fish, erratic swimming, and respiratory distress were observed. The implication of those findings in relation to environmental pollution is discussed.

Key words: Hepsetus odoe, Azadirachta indica, acute toxicity, extract.

INTRODUCTION

Aquatic pollution problems are due to the addition of deoxygenating substance, eroded sediments and chemical contaminants, urban Sewage, agriculture, wastes from industrial activities, radionuclides, salinization and acidification of soils, heated effluents from power plants. Recent assessments by the UNEP/WHO Global Environmental Monitoring System (GEMS) have identified the main water pollutants including sewage nutrients and toxic; metals as well as agricultural chemicals (UNEP, 1991). The most common water pollutants is organic material from domestic sewage, municipal waste, and agro-industrial effluent: (Lean et al, 1990).

Discharge of urban sewage and industrial and agro industrial waste waters rich in organic matter, such as effluents derived from pulp and paper production, palm oil extraction and sugar-beet processing containing high levels of decomposable organic substance; can lead to reduction of the oxygen content of recipient waters and create anoxic conditions in poorly mixed waters. The release of human and animal organic wastes carries the risk of high microbial loading which may lead to bacterial and viral infections (Barg et al 1996).

Water quality is deteriorating in key basin from urban agricultural and industrial wastes (WR 1990). Hazadous, sometimes toxic, trace elements are accidentally or deliberately dumped into water bodies. Pesticides and herbicides in drainage water and agricultural runoff from field are becoming a widespread source of aquatic pollution, and enclosed water bodies such as lakes, reservoirs, small water bodies and ponds are particularly at risk. Fish species and other aquatic biota which are top predators in the food chain may accumulate high levels of toxic compounds; in their bodies tissues which may well present a health hazard to humans if consumed in sufficient quality (Calamari and Naeve 1994).

Over the last years in many African countries the use of plant extract to catch fish has been on the increase. This has entailed a great increase in

discharge of pollutants to receiving waters causing undesirable effects on the aquatic environment and on fisheries.

The introduction of pollutants into the aquatic environment set in motion several disruptions to aquatic productivity which, according to Warren (1977), will eventually lead to some physiological dysfunctions in aquatic organisms. The use of plant extract for the control of aquatic molluses responsible for the transmission of water borne diseases has been documented (Osuala; 1989) Oli and Ukpabi (2000). Though there are advantages in the use of plant extracts in the control of aquatic snail vectors, the issue of pollution of the aquatic environment by such extracts, particularly their effects on fish, has been highlighted by Ufodike andl omoregie (1994). Medina and Woodbury (1979) screened members of the families Solanaceae, Fabiaceae, Rubiacene, and Euphorbiaceae for molluscicidae activity and found them to be potent on most snail vectors and other aquatic organisms. Ade-Serrano (1982) observed that the leaves, fruits, seeds, roots, and bark of the Neem plant, Azadirachta indica (Lodd) contain eriterperenoid, azatin, soslarin, and other active components with properties that are repellent and growth disruptive to living organisms. Omoregie and Okpanachi (1992), Oti and Ukpabi (2000) reckported growth retardation in Tilapia zilli (Gervais) and Heteroclaria exposed to sublethal concentrations of A. indica, and bark of Thevetia Peruviana respectively. Extracts of plants have been observed by Reed et al, (1969) to be used by fishermen in the tropics for fishing. Crude extracts are broadcast over the water surface, poisoning the fish which are then picked by hand from the water.

H. Odoe is a common member of the African tropical freshwater ichthyolfauna, where it is well suited as fish food (Anthony 1982), hence its choice for this investigation. Owing to the possible contamination of the tropical freshwater system with extracts of toxic plants and the death of information on their toxicity to tropical freshwater fish, the aim of the study was to evaluate the acute tolxicity of water extracts of the bark of A. indica (popular known as Dogon-yareo in Nigeria) to the African River Pike,

MATERIAL AND METHOD

Fingerlings of H. odoe (mean weight 6.01±0.10 g) of the same broodstock collected from Cross River in Nigeria were used for the investigation. The fish were acclimatized for 10 days in 20-litre glass aquaria, during which time they were fed twice daily at the ratwe of 4% of their body weight. Each aquarium was supplied with dechlorinated, well-aerated municipal tap water, which was changed daily. Mortality during the acclimation period was less than 2%. Thereafter the fish were stocked at 10 fish per aquarium for the experimental runs. Two aquaria acting as replications for each treatment (concentrations) were set up.

The bark of A. Indica was removed and dried at 60°C for 5 days. The well-dried samples were pounded in a clean mortar and sieved using a 0.1mm sieve. The fine particulate was dissolved in distilled water for 24 h at room temperature. (22.5°C). The settled aqueous portion was then decanted and centrifuged for 10 min at 1,500 rpm to remove particulate matter. The clear solution was filtered through a Whatman filter paper (No. 1) using a vacuum pump. The filtered solution was freeze-dried for 24 h. A stock solution of 100mg L^{-1} of the freeze-dried materials was made from which the following concentrations were prepared and introduced into each of the experimental aquaria along with their replication: 50, 25, 12.5, and 3.125 mg L^{-1}. The water in each

of the test aquaria was changed daily during which period fresh concentrations of the bark extract were introduced.

Methods for acute toxicity tests as described by Sprague (1973) were employed. The estimation of 96 h LC50 for the extract was determined by probit analysis using the arithmetical method. Mortality was recorded every 24 h though the aquaria were inspected every 3 h for dead fish, which were removed immediately. The opercular ventilation rate per minute was read at the start and every 24 h thereafter. During the exposure period the temperature, dissooved oxygen, free carbon dioxide, and pH in each aquarium were monitored every 4 h using methods described by APHA (1980). The temperature, dissolved oxygen, free carbon dioxide and pH of the experimental aquaria were observed to be maintained at the following ranges: 24.22-24.76°C, 7.27-7.40 mg L^{-1}, 2.39-2.52 mg L^{-1} and 6.48-6.55, respectively. The results obtained were subjected to statistical analysis with the application of Duncan's multiple range F-test for significant differences ($P<0.05$) between the various treatments.

RESULTS

Percentage, mortality decreased significantly ($P<0.05$) with decreasing concentrations of the extract (Table 1). No mortality was recorded in the control, group throughout the exposure period. The 96 h LC50 value was observed to be 8.00 mgL^{-1}, with lower and upper limits of 5.00 and 7.00 mgL^{-1} respectively. The computed regression equation was found to be $y = 5.50 + 1.70x$ ($r^2=0.900$).

Table 1: Mortality rate of Hepsetus odoe (Bloch) exposed to various acute concentrations of water extracts of the bark of Azadirachta indica (Lodd) results of two replications

Concentration(mgL^{-1})	Number of fish dead at				Range of mortality (%)
	24h	48h	72h	96h	
50.0	6	9	17	18	80 - 100
15.0	4	8	9	17	70 - 90
12.5	4	5	6	15	60 - 80
6.25		2	5	10	40 - 60
3.125		1	4	3	10 - 30
Control					0

At the higher concentrations, the colour of the exposed fish became darker, respiratory distress and erratic swimming being observed before death occurred. At lower concentrations (6.25 mgL^{-1} and less), such changes were minimal; the control group of fish showed no such signs.

The opercular ventilation rate of the exposed fish initially increased sharply, the increase being directly proportional ($P<0.05$) to the extract concentration (Table II). The opercular ventilation then decreased steadily until the 72 h of exposure. However, by the 96 h of exposure, the ventilation increased significantly ($P<0.05$) in the group of fish exposed to 12.5, 6.25 and 3.125 MgL^{-1}. The changes in the opercular ventilation rate of the control group of fish throughout the experiment were statistically non-significant.

Table II: Opercular ventilation (mean of six readings ± SE) rates per minute of Hepsetus odoe (Bloch) exposed to various acute concentration of Azadirachfa Indica (Lodd)

Concentration (mgL^{-1})	Time				
	Start	24 h	48 h	72 h	96 h
50.0	150±2.50	122±0.20	108±0.70	109±1.30	100±1`.20
25.0	143±1.70	119±0.20	102±0.20	98±0.90	100±1.02
12.5	130±0.92	90±1.30	90±1.30	98±1.10	105±0.90
6.25	100±0.50	102±0.50	90±1.70	98±0.80	102±1.19
3.125	90±0.37	92±0.10	90±0.82	92±1.10	102±1.20
Control	87±0.25	86±0.60	851.13	82±1.10	821.42

DISCUSSION

The results obtained from this study shows that concentrations of water extracts of the bark of A. Indica led to increased mortality of H. odoe. The value of 96 h LC50 of 8.00 mgL^{-1} reported in this work is much higher than those earlier reported by ufodike and Omoregie (1994). Oti and Ukpabi (2000) when they exposed the Nile tilapia Oreochromis niloticus (Trewavas) to water extracts of the bark of Balanites Aegyptiaca (Lodd) and Kigelia africana (Lodd). This shows that the bark extract of A. Indica is less toxic than those of B. Aegyptiaca and K. Africana, hence higher concentrations of A. Indica would be required to elicit 50% mortality of fish than the latter extracts. Osuala (1989) had earlier reported thata extracts of bark of A. Indica contain properties that are inhibitory to the normal metabolic processes of fish. Omoregie and Okpanachi (1992) reported retardation in the growth of T. zilli when exposed to sublethal concentrations of water extracts of bark of A. Indica. The increased mortality reported in the present investigation was due to the impairment of normal metabolism by the inhibitory components in the extracts. The darkening of the fish, respiratory distress, the initial hyperventilation, and erratic swimming observed in this study are indications that mortality of the exposed fish is not due to impaired metabolism but could in addition be due to nervous disorder. These abnormal behavioural responses in fish exposedk to toxicants were earlier reported by De silva and Ranashinghe, (1989) Ufodike and Omoregie (1990, 1994) and Okwusa and Omoregie (1995). It has been reported by many investigators that the dark colour of fish is due tos the dispersion of melanin pigments in the chromatophores which move towards the periphery, by pituitary hormone intermedin or M.S.H Novales (1959) reported that melanindispersion by M.S.H. requires the presence of Na^+ ions in the medium. He also stated that among the substances which afafect pigment movements in chromatophores, melanocyte stimulating hormone (MSH) has been most important. The extract also penetrate the non-scaly region very rapidly.

The skin membrane of the boarder of the operculum and over the skin on the rim of the eye is very thin. So any change in chromatophores will be seen very clearly. Fuji (1961) among others stated that melanophores are primarily controlled by the autonomic nervous system and the nerves which function are sympathetic. Distress and decrease in fish activity (metabolic) is due to depletion in energy substances like protein, carbohydrates and lipids during pollution. The

reduction in metabolic aactivity with increasing concentration of the extract may have been due to the increased utilization of the body substances (Carbohydrates protein, and lipids) during stress (Kabeer Ahmed et al, 1979). .Similar results are also reported in fish by various authors. Umminger (1970) is of the opinion thata carbohydrates represents the principal and immediate energy precursors of animals when exposed to stress conditions, where protein being energy source of spare during chronic condition of stress. Various authors had similar reports on fish exposed to toxicants (Oti and Ukpabi 2000, Jayachandra and Chockalingam 1986). Decrease in fish activity in this present investigation indicates the utilization of all three substances when the fish is under stress. If the principal and immediate energy source gets depleted, the other sources exhibit a proportional depletion as the metabolism of these substances are inter-linked through a common metabolic pathway i.e tricarboxylic acid Cycle (TCA). Hence there is a depletion in all the component of fish tissues leading to stress.

The use of extracts of A. Indica and other similar plants by local fishermen in rivers, stream and lakes, coupled with their use in the control of aquatic snail reservoirs vectors, is seriously ill advised, as the resultant deleterious effects on fish subjected to acute exposure will subsequently lead to death. If the present rate at which these extracts are being used in Africa is not checked, the continuous existence of the aquatic fauna, including biologically important fish species, will be in serious jeopardy. The environment authorities within the tropical region of the world, need to set new quality standard on the use of these plant extracts in the aquatic environment.

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